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A composite polymer coated sorbent with a bidisperse pore size distribution for the simultaneous separation and desalting of bioploymers

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A composite polymer-coated sorbent with a bidisperse pore size distribution for the simultaneous separation and desalting of biopolymers

The invention is concerned with the use of a composite sorbent comprising a support, which is at least partially covered by a polymeric film based on polyanilin or other primary aromatic amines, as it has been described in EP 00 934 960.6. Such sorbents have been succesfully used for a very rapid and convenient separation of DNA from other bio-macromolecules. The disadvantage of the technical solution with respect to a extremly accelerated one-step procedure for DNA purification is the rather pure retention of low molecular weight compounds in cell lysates, which usually represent a great amount of inhibitors of DNA modifying enzymes.

The technical problem underlying the present invention is to provide a method and material by which additionally to the separation of biomacromolecules on polyanilin surfaces low molecular weight substances can be efficiently retained on the sorbent.

The technical problem is solved by the use of a support for the polymeric surface modification, which has a porous structure with a bidisperse or oligo-disperse distribution of pore sizes. Such structures build the basis for sorbent materials according to the present invention, which allow additionally to the separation of bio-macromolecules the improved retention of low molecular weight substances.

Preferably the support is an inorganic material selected from the groups comprising inorganic metal oxides preferably with a porous structure such as oxides of aluminium, titanium, zirconium, silicon and/or iron.

In a further embodiment of the invention the support is an organic material preferably with a porous structure such as linear or cross-linked polystyrene,

polyethylene, polyacrylate particles or surfaces.

Preferably, the support containing inorganic or organic materials is in particle-like or monolithic membrane-like form and has a porous structure which shows a bidisperse or oligodisperse distribution of pore sizes. Such structures build, e. g., the basis for sorbent materials according to the present invention, which allow additionally to the separation of bio-macromolecules such as nucleic acids or proteins the improved retention of low molecular weight substances having, e. g., molecular weights of less than 500 Da. Such bidisperse supports may preferentially be obtained by means of gelling (gel building) of silica sols, starting the process with the mixture of two size types of monodisperse colloidal silica particles. The mass proportion of these two types of colloidal particles determines the proportion and distribution of differently sized pores in the final silica support material.

Typically, two types of silica sols are prestructured prior to mixing. Prestructuring occurs, e.g., by temperature treatment or other methods and partially evaporating water. The ratio of the mean diameter of the large pore size distribution and the lower pore size distribution is in the range of 3-15, in particular 4-10. The mean diameter of the larger pore size distribution should not be smaller than 25 to 50 nm and should not exceed 2000 nm, in another embodiment 1000 nm.

The composite sorbent with a bidisperse or oligodisperse distribution of pore sizes has preferentially an at least partial coating on the support, which coating comprises essentially polyanilines or derivatives of polyanilines for the separation, isolation, identification, purification (e.g. desalting) and/or detection of biomolecules in particular nucleic acids, proteins, polysaccharides in analytical or preparative scale.

The derivatives of polyaniline are preferably substituted or nonsubstituted alkyl anilines, aromatic systems, ethylaniline, propylanilin, and/or ethoxyanilin.

The main advantages of the use of the composite sorbent as suggested earlier [A. Syed et al. "Polyaniline: Reaction Stoichiometry and Use as an Ionexchange Polymer and Acid/Base Indicator"; *Synthetic Metals*, 36, 209-215(1990); US Patent 5,281,363; 1/1994, Shacklette et al.; 252/500; US Patent 5,232,631; 8/1993, Yong Cao et al.; 252/500; J. Nicolau "Characteristic of Polyaniline filled by Porouse n^+-type Silica by the Renthgene Photoelectrical Specthroscopy Method"; *Synthetic Metals*, 1-3, 2073-2074(1995)] are the ease of handling, the speed of the separation process and the possibility of a visual control of sorption and separation processes. DNA is contained in the flow-through (cartridge methods) or in the supernatant (batch methods). Low molecular weight substances are retained in the column and can be washed of separatly from the DNA fraction. Bound proteins can be eluted separately by a gradient and subsequently analysed if needed.

The present invention is further illustrated by the following examples, which are understood to be not limiting.

Example 1:

For the synthesis of sorbents with a bidisperse or oligodisperse distribution of pore sizes, a silica gel has been prepared in the following way:

The two starting types of silica sol in water had following characteristics:

A: particle diameter: 6 nm; SiO_2 concentration: 22 mass %; Na^+- stabilised pH: 9.1

B: particle diameter: 40 nm; SiO_2 concentration: 40 mass %; Na^+- stabilised pH: 9.2

Water from the two silica sols was evaporated at pH 5.0 in a water bath at 80 °C by constant stirring until 30 and 60 mass %, respectively. To 100 ml of sol A structured by evaporation were added 50 ml of structured sol B and the

evaporation has been continued until the formation of a homogeneous gel. The silica hydrogel obtained after 4 hours sinerethis (partial shrinkage) was dried, first for 4 hours at 80 °C in a water bath, followed by 3 hours at 130 °C in a drying hood. Afterwards the product was treated at 600 °C for 5 hours in a muffel oven. The ready obtained silica gel was grinded, fractionated and analysed for pore size distribution both by mercury porometry (according to DIN 66 133 (1993)) and BET-method (according to ISO 9277). These analyses showed a preferential pore size in two classes of 5 nm and 28 nm, a sorption volume of 0.7 cm^3/gr and a specific surface of 120 m^2/gr.

Example 2:

The two starting types of silica sol in water had following characteristics:

A: particle diameter: 10 nm; SiO_2 concentration: 30 mass %; Na^+- stabilised pH: 9.2

B. particle diameter: 80 nm; SiO_2 concentration: 50 mass %; Na^+- stabilised pH: 9.1

The silica gel sorbent was prepared as in example 1, with following variations:

Water from the two silica sols was evaporated at pH 4.5 in a water bath at 80 °C by constant stirring until 52 and 60 mass %, respectively. To 100 ml of sol A structured by evaporation were added 130 ml of structured sol B. Analyses showed a preferential pore size in two classes of 7 nm and 60 nm, a sorption volume of 0.75 cm^3/gr and a specific surface of 95 m^2/gr.

Example 3:

An amount of 2.5 to 15 g of the porous support as described in Example 1 is transferred to a glass ampoule (reaction vessel). The ampoule is connected to a vacuum source and via a valve to a reservoir containing 30 ml of an aqueous solution of the monomers (1-4 $\mu l/m^2_{CPGsurface}$) and dopant (HCl) in molar ratio

of 1:3. A vacuum of 10 mbar is applied to the ampoule. When the support particles stopped moving (after approx. 20 min.) the monomer-containing solution is filled into the ampoule from the bottom while the line to the vacuum source is closed. During this step the monomer solution is wetting the support and penetrating into the pores of the support particles. Now the valve to the monomer reservoir is closed. The next step has to be carried out as quick as possible. Under atmospheric pressure 20 ml of an oxidizer solution (0,005 - 0.008 $g/1m^2_{CPGsurface}$) are added into the ampoule under mixing. The mixing is continued at 20°C for 0.5-3 h. After the first 10-15 min the suspension colour becomes dark-blue. At the end of the process the colour of the suspension changes to dark-green. The product is removed from the ampoule and then washed with water (40 ml per gram) and then incubated in 1M ammonium hydroxide (20 ml per gram) for 3 h. The colour of the composite sorbent becomes dark-blue. After that the sorbent is extensively washed with 50 %-Vol. Methanol in water (100 ml per gram) and dried at 60°C in a vacuum oven for 17 h.

Example 4:

The described modified sorbents were used for the purification of genomic DNA from lysates of *Escherichia coli.*

1. An overnight culture was made from the strain *E. coli JM 109* (50 µl bacteria cells, 10 ml medium, 37°C).

2. From this culture 1 ml was centrifugated in micro centrifuge tubes.

3. After removal of the supernatant the bacterial pellet was suspended in 100 µl buffer 1 (2 mg/ml lysozyme, 2 mM $CaCl_2$, 100 mM Tris-HCl pH 7.9, 4% succrose).

4. For cell lysis the suspension was incubated for 8 min at 60°C.

5. 100 µl buffer 2 were added (1% MIRA Tensid-Mix, 1.5 mM EDTA) and

cooled to room temperature.

6. The mixture was shaken 10 min at room temperature and incubated for further 5 min without shaking at room temperature.

7. The mixture was centrifuged for 2 min at 13,000 rpm.

8. The supernatant was given onto a sorbent-packed column and eluted with TE-buffer.

Preparation and use of the sorbent

The sorbent is subsequently wetted in methanol, 50% methanol and water and then degassed for 0.5 h. The supernatant is decanted and the sorbent washed 4 times with TE buffer. While stirring the sorbent in TE buffer it is degassed under vacuo in an exciccator. Cartridges are packed with this suspension of the sorbent (120 mg/ml).

A bacterial lysate (see above, step 8) from 1 ml of overnight culture is prepared and pipetted onto the cartridge and eluted with TE buffer. The cartridge is incubated 5-10 min without elution. Five fractions with volume of 200 µl are collected immediatly after the cartridge starts to drop. The fractions are further analysed by agarose gel electrophoresis (0.8% agarose in 89 mM Tris; 89 mM boric acid; 2 mM EDTA) at a constant current of 100 mA.

Gels are stained with ethidium bromide. Genomic DNA but not RNA is found in the second fraction. The DNA contaning fraction is measured in a spectrophotometer. The ratio of the absorption A_{260}:A_{280} of such fractions is in the range of 1.58 to 1.78.

Claims

1. A composite polymer-coated sorbent with a bidisperse or oligodisperse distribution of pore sizes and having an at least partial coating on its surface, which coating comprises essentially polyanilines or derivatives of polyanilines and use thereof for the simultaneous separation and purification of bio-macromolecules.

2. The sorbent material according to claim 1 wherein the support is a porous inorganic material selected from the group comprising inorganic metal oxides, such as oxides of aluminium, titanium, zirconium, silicon oxides, and/or iron oxides.

3. The sorbent material according to claim 1 wherein the support is an organic material, preferably of porous structure such as cross-linked polystyrenes, polyacrylates, and polyethylenes.

4. The sorbent material according to claim 2, wherein the inorganic material with a bidisperse distribution of the pore sizes is obtainable by gelling a mixture of two silica sols with differently sized colloidal silica particles.

5. The sorbent material according to claims 1 to 4 wherein the support is in particle-like or monolithic membrane-like form.

6. The sorbent material according to claims 1 to 5, wherein the derivatives of polyaniline are substituted or nonsubstituted alkyl anilines, aromatic systems, ethylaniline, propylanilin, and/or ethoxyanilin.

Abstract

A composite polymer-coated sorbent with a bidisperse or oligodisperse distribution of pore sizes and having an at least partial coating on its surface, which coating comprises essentially polyanilines or derivatives of polyanilines and use thereof for the simultaneous separation and desalting of bio-macromolecules.